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Amplified electrochemiluminescent aptasensor using mimicking bi-enzyme nanocomplexes as signal enhancement

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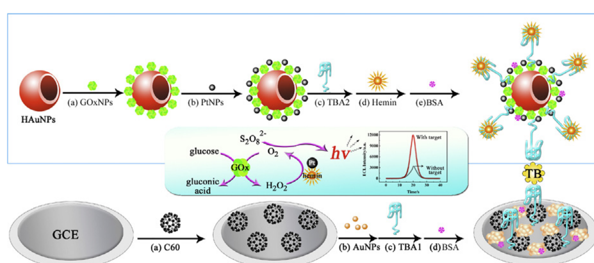
HIGHLIGHTS

- Mimicking bi-enzyme nanocomplexes was employed as a signal enhancer.
- C_{60} nanoparticles constructed a novel and effective sensitive interface.
- A sandwich-type electrochemiluminescence aptasensor for thrombin detection.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, a sandwich-type electrochemiluminescence (ECL) aptasensor for ultrasensitive detection of thrombin (TB) was designed based on mimicking bi-enzyme cascade catalysis to in situ generate coreactant of dissolved oxygen (O_2) for signal amplification. We utilized hollow Au nanoparticles (HAuNPs) as carriers to immobilize glucose oxidase nanoparticles (GOxNPs) and Pt nanoparticles (PtNPs) by electrostatic adsorption. Then, the detection aptamer of thrombin (TBA 2) was immobilized on the PtNPs/GOxNPs/HAuNPs nanocomplexes. Finally, hemin was intercalated into the TBA 2 to obtain the hemin/G-quadruplex structure. The hemin/G-quadruplex was an interesting DNAzyme that commonly mimiced horseradish peroxidase (HRP). Herein, GOxNPs, hemin/G-quadruplex and PtNPs could form mimicking bi-enzyme cascade catalysis system to in situ generate dissolved O_2 as coreactant in peroxydisulfate solution when the testing buffer contained proper amounts of glucose. This method had successfully overcome the disadvantage of difficulty to label the dissolved O_2 and realized the ECL signal amplification. The experiment proved that the aptasensor had good linear relationship on low concentration of TB. The linear range was 1×10^{-6}–10 nM, with a detection limit of 0.3 fM.

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1. Introduction

With the merits of electrochemical and luminescent techniques, electrochemiluminescence (ECL) analysis, has become a powerful analytical tool for highly sensitive and specific detection of clinical samples [1–3]. In the past several decades, peroxydisulfate ($S_2O_8^{2-}$) is well known as a co-reactant in the ECL studies of $Ru(bpy)_3^{2+}$ [4], quantum dots [5] and silicon nanosphere [6]. Nowadays, the ECL of peroxydisulfate/oxygen ($S_2O_8^{2-}/O_2$) system in aqueous buffered solutions exhibits fascinating characteristic, since its advantages of simplicity, availability, sensitivity and cheapness [7,8]. According to the luminescence mechanism of $S_2O_8^{2-}$, it is found that the dissolved O_2 can serve as a desirable coreactant except it has the disadvantage of difficulty to label [9]. Thus, it is necessary for searching the solution to increase the concentration of the dissolved O_2 for enhancing the intensity of peroxydisulfate system.

In our previous works, we found that to in situ generate the dissolved O_2 via the enzymatic reaction was an effective approach.

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Thus, the bi-enzymatic reaction of glucose oxidase (GOx) and horseradish peroxidase (HRP) [10] or mimic bi-enzyme system by GOx and hollow PtPd bimetal alloy nanoparticles (HPtPd) [11] were all commendable methods to enhance the ECL sensitivity of peroxydisulfate system. Herein, we explored another enzymatic reaction to in situ generate dissolved O_2. Hemin is a well-known natural metalloporphyrin and it can condense with a hydroxyl or amino group in protein, which reveals peroxidase-like activities [12,13]. Moreover, recent research indicates that hemin is a kind of newly discovered HRP mimicking enzyme, which shows significant catalysis to H_2O_2 [14–16]. In our previous work, we had constructed an ECL sensing platform utilizing hemin-graphene nanosheets as ECL amplification and sensing element based on luminol [17]. Thus, the introduction of HRP-mimicking enzyme of hemin to the fabrication of an aptasensor is of great value.

In recent years, carbon nanomaterials, especially two-dimensional graphene and one-dimensional carbon nanotubes (CNTs) have been widely used to construct biosensing interface [18,19]. C_{60}, with conjugate π electron structure, is a truncated icosahedron made out of five and six member rings of sp^2 carbons [20]. It was also found that C_{60} nanoparticles (nano-C_{60}) could serve as a novel, effective, fluorescent sensing platform for biomolecular detection with high sensitivity and selectivity [21,22]. Therefore, we utilized the zero-dimensional nano-C_{60} to construct a novel and effective sensitive interface and it not only enhanced the immobilization of nanoparticles but also amplified the ECL signal owing to its large specific surface area.

In the present work, a novel sandwich-type aptasensor was developed based on mimicking bi-enzyme cascade catalysis to in situ generate coreactant of dissolved O_2 for signal amplification to detect thrombin (TB). We utilized hollow Au nanoparticles (HAuNPs) as carriers to immobilize glucose oxidase nanoparticles (GOxNPs) and Pt nanoparticles (PtNPs). Then, the detection aptamer of thrombin (TBA 2) was immobilized on the PtNPs/GOxNPs/HAuNPs and hemin was intercalated into the TBA 2 to obtain the hemin/G-quadruplex/PtNPs/GOxNPs/HAuNPs nanocomplexes, which was utilized as signal tags. Besides, nano-C_{60} and electrochemical deposited Au nanopaticles as a nanomatrix was sequentially constructed on the surface of glassy carbon electrode (GCE) for further immobilization of thiol-terminated thrombin capture aptamer (TBA 1). During the detection process, the TBA 1, TB, and TBA 2 make a sandwich-type structure. When proper amounts of glucose were added in the peroxydisulfate solution, GOxNPs could catalyze the glucose to generate H_2O_2 which could be further catalyzed by hemin/G-quadruplex and PtNPs to in situ generate dissolved O_2 of high concentration, resulting in a considerably enhancement of ECL signal.

2. Experimental

2.1. Reagent

Fullerene C_{60} (99.5%) was obtained in Pioneer Nanotechnology Co. (Nanjing, China). The sequence of capture thrombin aptamer (TBA 1, 2.5 μM) is as follows: 5′-SH-$(CH_2)_6$-GGTTGGTGTGGTTGG-3′, the sequence of detection thrombin aptamer (TBA 2, 2.5 μM): 5′-NH_2-$(CH_2)_6$-GGTTGGTGTGGTTGG-3′, which were obtained from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China). Thrombin (TB), bovine serum albumin (BSA), hemin, hemoglobin (Hb), gold chloride ($HAuCl_4$), 3-thiophenemalonic acid (TA), chloroplatinic acid (H_2PtCl_6) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Glucose oxidase (GOx) was obtained from the Shanghai Biochemical Co. (China). $Na_2S_2O_8$ was purchased from Chengdu Chemical Reagent Company (Chengdu, China). Phosphate buffered solution (PBS) (pH 7.4) was prepared using 0.1 M Na_2HPO_4, 0.1 M KH_2PO_4 and 0.1 M NaCl. 20 mM Tris–HCl buffer (pH 7.4) containing 140 mM NaCl, 5 mM KCl and 1 mM $MgCl_2$ was used to prepare aptamer solutions. 10 mM $K_3Fe(CN)_6$, 10 mM $K_4Fe(CN)_6$, 0.1 M Na_2HPO_4, 0.1 M KH_2PO_4, and 0.1 M KCl were used to prepare $[Fe(CN)_6]^{3-/4-}$ solution. The serum specimens were obtained from Southwest Hospital. All other chemicals were of analytical grade and used as received. Double distilled water was used throughout this study.

2.2. Apparatus

The ECL emission was monitored with a model MPI-A electro-cheminescence analyzer (Xi'an Remax Electronicscience & Technology Co. Ltd., Xi'an, China) with the voltage of the photomultiplier tube (PTM) set at 800 V and the potential scan from 0 to 2.0 V in the process of detection. Cyclic voltammetric (CV) and electrochemical impedance spectroscopy (EIS) measurements were performed with a CHI 610 A electrochemistry workstation (Shanghai CH Instruments, China). The experiment was performed with a conventional three-electrode system, in which the modified glassy carbon electrode (GCE) was the working electrode, a platinum wire was the counter electrode and an Ag/AgCl (sat.KCl) was the reference electrode. JBZ-12H Electromagnetic Stirrer was used in the synthesis of hemin/G-quadruplex/PtNPs/GOxNPs/HAuNPs nanocomplexes. The morphologies of nanoparticles were estimated from a transmission electron microscopy (TEM, H600, Hitachi Instrument, Japan) and a scanning electron microscope (SEM, S-4800, Hitachi Instrument, Japan).

2.3. Pretreatment of nano-C_{60}

The nano-C_{60} was prepared according to the literature with minor modification [23]. First of all, appropriate amount of C_{60} powder was dispersed in 2 mL toluene with the aid of ultrasonic agitation. Then, 2 mL distilled water was added into the solution and standed until the solution was layered obviously. After successive sonication, toluene in the solution would completely volatilize. Afterwards, the yellow product was collected through centrifugation, the upper solution was removed and the lower product was washed three times with distilled water. At last, the collected lower sediment was dispersed in distilled water until use.

2.4. Preparation of HAuNPs

According to the literature, the HAuNPs was synthesized with some modification [24]. Firstly, the containers were soaked in chromic acid solution for cleaning, then 190 μL $C_6H_5Na_3O_7{\cdot}2H_2O$ (0.1 M) and 400 μL freshly prepared $NaBH_4$ (1 M) were added in 100 mL distilled water under an N_2 purging condition. Afterwards, 100 μL $CoCl_2{\cdot}6H_2O$ (0.5 M) was added into the solution with stirring for 15–60 min when the color of the solution turned from dark pink to brown gray. After bubbles in the solution completely stopping, 50 μL $HAuCl_4$ (0.1 M) was added into the solution for six times under stirring. At last, the bluish mixture was allowed to react in the air for 30 min under stirring without N_2 purging to oxidize the remaining Co nanoparticles.

2.5. Preparation of GOxNPs

The GOxNPs was prepared according to the published method with some modification [25]. Firstly, 2 mg freshly prepared GOx was added in 1 mL deionized water. Then, at room temperature, 4 mL ethanol was added dropwise into the solution under stirring. Afterwards, 0.5 μL glutaraldehyde (8%) was added into the solution with stirring in the ice-water bath for 24 h. In addition, 0.1 g cysteine was added into the mixture under stirring for 4 h. Finally, the

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